



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No.: **WARF-0002** **RECEIVE**  
Inventors: **Allen S. Laughon** APR 12 2002  
Serial No.: **09/810,385** TECH CENTER 1600/2000  
Filing Date: **March 16, 2001**  
Examiner: **Not Assigned**  
Group Art Unit: **1641**  
Title: **Compositions and Methods for Negative Regulation of TGF- $\beta$  Pathways**

"Express Mail" Label No. **EV106519661US**  
Date of Deposit - April 9, 2002

I hereby certify that this paper is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the U.S. Patent and Trademark Office, Box Sequence, P.O. Box 2327 Arlington, VA 22202.

By Jane Massey Licata  
Typed Name: **Jane Massey Licata, Reg. No. 32,257**

U.S. Patent & Trademark Office  
Box Sequence, P.O. Box 2327  
Arlington, VA 22202

Dear Sir:

**PRELIMINARY AMENDMENT**

Please amend the first paragraph on page 2, beginning at line 1, as follows:

Genetic and biochemical studies indicate that TGF- $\beta$  and its related factors, including activin, bone morphogenetic proteins (BMPs), and their *Drosophila* counterpart, decapentaplegic, each signal to their target cells by a unique signaling cascade activated by ligand-induced serine/threonine kinase receptor

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 2

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

complex formation (Wrana, J. 1998. *Miner. Electrolyte Metab.* 24:120-130). It is now well established that TGF- $\beta$  signaling pathways switch target genes on through the activities of Smad proteins. These cytosolic proteins are recruited and phosphorylated by the TGF- $\beta$ , activin, or BMP receptor complexes. Smad proteins exist as monomers in unstimulated cells but homo- or hetero-dimerize and translocate to the nucleus of the cells where they then activate target gene expression through contact with cofactors and DNA.

Please amend the second paragraph beginning on page 2, line 16, as follows:

Thus, although much is now known about how TGF- $\beta$  pathways switch genes on, little is known about how genes can be switched off. There are examples of such negative regulation in vertebrates and in model organisms such as *C. elegans* and *Drosophila*. In mammals, growth inhibition by TGF- $\beta$  is correlated with negative regulation of *c-myc* and *cyclin A* (Feng, X.H. et al. 1995. *J. Biol. Chem.* 270:24237-24245). TGF- $\beta$  also negatively regulates proteases that degrade components of the extracellular matrix such as collagen (Kerr, L.D. et al. 1990. *Cell* 61:267-278). Evidence that Smad proteins can directly repress or

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 3

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

negatively regulate transcription comes from genetic analysis of the *C. elegans* TGF- $\beta$  pathway that regulates choice between reproductive growth and diapause (Patterson, G.I. et al. 1997. *Genes Develop.* 11:2679-2690). Activation of this pathway overrides negative regulation by the Smad4-related Daf-3 protein. Negative regulation by Smad proteins was also shown in *Drosophila*, where the *Drosophila* BMP4 homolog, decapentaplegic (dpp), was shown to activate its targets by repressing expression of a novel repressor known as Brinker (Campbell, G. And A. Tomlinson. 1999. *Cell* 96:553-562; Jazwinska, A. Et al. 1999. *Cell* 96:563-573; Minami, M. Et al. 1999. *Nature* 398:242-246; Sivasankaran, R. Et al. 2000. *EMBO J.* 19:6162-6172). Ectopically expressed Brinker was able to repress BMP targets in frog embryos as well, indicating that this double negative mechanism is likely to operate in vertebrates as well as in *Drosophila*. A second negatively regulated target is the segment polarity gene, wingless (wg), which is repressed in response to Dpp in the embryonic ectoderm (Grieder, N. et al. 1995. *Cell* 81:791-800) and in imaginal discs (Penton, A. and F.M. Hoffmann. 1996. *Nature* 382:162-165; Brook, W.J. and S.M. Cohen. 1996. *Science* 273:1373-1376; Jiang, J. and G. Struhl. 1996. *Cell*

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 4

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

86:401-409; Theisen, H. et al. 1996. *Development* 122:3939-3948;  
Tomoyasu, Y. et al. 1998. *Development* 125:4215-4224; Chanut, F.  
and U. Heberlein. 1997. *Development* 124:559-567).

Please amend the paragraph on page 3, beginning on line 20,  
as follows:

Although repression by TGF- $\beta$  pathways could be indirect,  
mounting evidence shows that Smad proteins interact directly with  
a variety of co-repressors. Smad proteins interact with the  
repressors Evi-1 (Kurokawa, M. Et al. 1998. *Nature* 394:92-96),  
Gli3 (Liu, F. Et al. 1998. *Nature Genet.* 20:325-326), TGIF  
(Wotton, D. Et al. 1999. *Cell* 97:29-39), SIP1 (Verschueren, K. Et  
al. 1999. *J. Biol. Chem.* 274:20489-20498), and the oncoproteins  
SKI (Luo, K.S. et al. 1999. *Genes Develop.* 13:2196-2206), SnoN  
(Stroschein, S.L. et al. 1999. *Science* 286:771-774), and  
adenovirus E1A (Nishihara, A. et al. 1999. *J. Biol. Chem.*  
274:28716-28723). It is known that some viruses inhibit cellular  
responses to TGF- $\beta$  and the finding that E1A interacts directly  
with Smad proteins supports this finding. Binding of Smad3 to  
E1A or TGIF inhibits Smad binding of the coactivator p300.  
Contact with TGIF or SKI recruits histone deacetylase and  
inhibits transcriptional activation by Smad2 and Smad3. Contact

Attorney Docket No.: **WARF-0002**  
Inventors: **Allen S. Laughon**  
Serial No.: **09/810,385**  
Filing Date: **March 16, 2001**  
Page 5

with Evi-1 inhibits DNA binding of Smad3. However, because Smad proteins are not known to have any intrinsic ability to function as repressors, and in fact have just the opposite effect, the function generally ascribed to DNA-binding Smad co-repressors is one of dampening of transcriptional activation by Smads, leaving the mechanism of TGF $\beta$ -induced repression unexplained. Until the present invention it was not appreciated that Smad proteins are able to interact directly with co-repressor genes through a specific Smad domain. Thus, the present invention describes the interaction between Smad proteins and the general co-repressor dCtBP and shows how this interaction provides a mechanism for the ability of activated Smads to directly repress transcription in response to signaling.

Please amend the paragraph beginning on page 6, line 10, as follows:

Figure 3 depicts the results of experiments examining the ability of dCtBP to inhibit activation of Smad box-lacZ reporter by Mad and Medea in Drosophila cells. The data shown are the average of three S2 transfections. Each LRR repeat contained three Smad boxes arranged as : AGAC GTCT GTCT (SEQ ID NO:1).

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 6

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

Please amend the paragraph on page 16, beginning at line 6,  
as follows:

LRR-lacZ and RLL-lacZ construction have been described by  
Johnson et al. (Johnson, K. et al. 1999. *J. Biol. Chem.*  
274:20709-20716).

**REMARKS**

This preliminary amendment is being filed to identify the  
sequences listed in Figure 8 by SEQ ID NO and the sequence  
identified in the description of the drawing for Figure 3.  
Inadvertent typographical errors in page numbers of some of the  
references are also being corrected. We are also enclosing  
formal drawings and a paper copy and computer readable copy of  
the sequence listing. No new matter has been added.

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 7

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

Attached hereto is a marked up version of the changes made to the specification by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

Respectfully submitted,

*Jane Massey Licata*  
Jane Massey Licata  
Registration No. 32,257

Date: **April 9, 2002**

Licata & Tyrrell P.C.  
66 Main Street  
Marlton, N.J. 08053  
856-810-1515

Attorney Docket No.: **WARF-0002**  
Inventors: **Allen S. Laughon**  
Serial No.: **09/810,385**  
Filing Date: **March 16, 2001**  
Page 8

**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the specification:**

Please amend the first paragraph on page 2, beginning at line 1, as follows:

Genetic and biochemical studies indicate that TGF- $\beta$  and its related factors, including activin, bone morphogenetic proteins (BMPs), and their *Drosophila* counterpart, decapentaplegic, each signal to their target cells by a unique signaling cascade activated by ligand-induced serine/threonine kinase receptor complex formation (Wrana, *J. Miner. Electrolyte Metab.* 24:~~1201-30~~ 120-130). It is now well established that TGF- $\beta$  signaling pathways switch target genes on through the activities of Smad proteins. These cytosolic proteins are recruited and phosphorylated by the TGF- $\beta$ , activin, or BMP receptor complexes. Smad proteins exist as monomers in unstimulated cells but homo- or hetero-dimerize and translocate to the nucleus of the cells where they then activate target gene expression through contact with cofactors and DNA.

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 9

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

Please amend the second paragraph beginning on page 2, line 16, as follows:

Thus, although much is now known about how TGF- $\beta$  pathways switch genes on, little is known about how genes can be switched off. There are examples of such negative regulation in vertebrates and in model organisms such as *C. elegans* and *Drosophila*. In mammals, growth inhibition by TGF- $\beta$  is correlated with negative regulation of *c-myc* and *cyclin A* (Feng, X.H. et al. 1995. *J. Biol. Chem.* 270:24237-24245). TGF- $\beta$  also negatively regulates proteases that degrade components of the extracellular matrix such as collagen (Kerr, L.D. et al. 1990. *Cell* 61:267-278). Evidence that Smad proteins can directly repress or negatively regulate transcription comes from genetic analysis of the *C. elegans* TGF- $\beta$  pathway that regulates choice between reproductive growth and diapause (Patterson, G.I. et al. 1997. *Genes Develop.* 11:2679-2690). Activation of this pathway overrides negative regulation by the Smad4-related Daf-3 protein. Negative regulation by Smad proteins was also shown in *Drosophila*, where the *Drosophila* BMP4 homolog, decapentaplegic (dpp), was shown to activate its targets by repressing expression of a novel repressor known as Brinker (Campbell, G. And A.

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 10

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

Tomlinson. 1999. *Cell* 96:553-562; Jazwinska, A. Et al. 1999.

*Cell* 96:563-573; Minami, M. Et al. 1999. *Nature* 398:242-246;

Sivasankaran, R. Et al. 2000. *EMBO J.* 19:6162-6172).

Ectopically expressed Brinker was able to repress BMP targets in frog embryos as well, indicating that this double negative mechanism is likely to operate in vertebrates as well as in *Drosophila*. A second negatively regulated target is the segment polarity gene, wingless (wg), which is repressed in response to Dpp in the embryonic ectoderm (Grieder, N. et al. 1995. *Cell* 81:791-800) and in imaginal discs (Penton, A. and F.M. Hoffmann. 1996. *Nature* 382:162-~~164~~ 165; Brook, W.J. and S.M. Cohen. 1996. *Science* 273:1373-~~1377~~ 1376; Jiang, J. and G. Struhl. 1996. *Cell* 86:401-409; Theisen, H. et al. 1996. *Development* 122:3939-3948; Tomoyasu, Y. et al. 1998. *Development* 125:4215-4224; Chanut, F. and U. Heberlein. 1997. *Development* 124:559-567).

Please amend the paragraph beginning on page 3, line 20, as follows:

Although repression by TGF- $\beta$  pathways could be indirect, mounting evidence shows that Smad proteins interact directly with a variety of co-repressors. Smad proteins interact with the repressors Evi-1 (Kurokawa, M. Et al. 1998. *Nature* 394:92-96),

Attorney Docket No.: **WARF-0002**  
Inventors: **Allen S. Laughon**  
Serial No.: **09/810,385**  
Filing Date: **March 16, 2001**  
Page 11

Gli3 (Liu, F. Et al. 1998. *Nature Genet.* 20:325-326), TGIF (Wotton, D. Et al. 1999. *Cell* 97:29-39), SIP1 (Verschueren, K. Et al. 1999. *J. Biol. Chem.* 274:20489-20498), and the oncoproteins SKI (Luo, K.S. et al. 1999. *Genes Develop.* 13:2196-2206), SnoN (Stroschein, S.L. et al. 1999. *Science* 286:771-779 774), and adenovirus E1A (Nishihara, A. et al. 1999. *J. Biol. Chem.* 274:28716-28723). It is known that some viruses inhibit cellular responses to TGF- $\beta$  and the finding that E1A interacts directly with Smad proteins supports this finding. Binding of Smad3 to E1A or TGIF inhibits Smad binding of the coactivator p300. Contact with TGIF or SKI recruits histone deacetylase and inhibits transcriptional activation by Smad2 and Smad3. Contact with Evi-1 inhibits DNA binding of Smad3. However, because Smad proteins are not known to have any intrinsic ability to function as repressors, and in fact have just the opposite effect, the function generally ascribed to DNA-binding Smad co-repressors is one of dampening of transcriptional activation by Smads, leaving the mechanism of TGF $\beta$ -induced repression unexplained. Until the present invention it was not appreciated that Smad proteins are able to interact directly with co-repressor genes through a specific Smad domain. Thus, the present invention describes the

Attorney Docket No.:  
Inventors:  
Serial No.:  
Filing Date:  
Page 12

**WARF-0002**  
**Allen S. Laughon**  
**09/810,385**  
**March 16, 2001**

interaction between Smad proteins and the general co-repressor dCtBP and shows how this interaction provides a mechanism for the ability of activated Smads to directly repress transcription in response to signaling.

Please amend the paragraph beginning on page 6, line 10, as follows:

Figure 3 depicts the results of experiments examining the ability of dCtBP to inhibit activation of Smad box-lacZ reporter by Mad and Medea in Drosophila cells. The data shown are the average of three S2 transfections. Each LRR repeat contained three Smad boxes arranged as : AGAC GTCT GTCT (SEQ ID NO:1).

Please amend the paragraph on page 16, beginning at line 6, as follows:

LRR-lacZ and RLL-lacZ construction have been described by Johnson et al. (Johnson, K. et al. 1999. *J. Biol. Chem.* 274:20709-20726 20716).